

US EPA ARCHIVE DOCUMENT

GREENSBORO, N.C.

PAGE 1 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION 11/25/80		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:



## 1.0 SCOPE

This total residue method is used for the determination of the combined residues of metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester] and its metabolites which contain the 2,6-dimethylaniline moiety in crop samples. (See Figure 1 for structures). This method is a modification of Analytical Method AG-330 developed originally for tobacco. The limit of detection for the method is 0.05 ppm expressed in metalaxyl equivalents.

## 2.0 PRINCIPLE

Crop samples with high moisture content (e.g., potatoes, lettuce, cabbage, fruit crops, broccoli, etc.) are extracted by blending with 20% water/methanol for 10 minutes. Dry crop samples (e.g., cottonseed, soybeans, etc.) are extracted by refluxing with 20% water/methanol for two hours. Cottonseed extracts are partitioned between acetonitrile and hexane to remove the oils and fats which cause interferences in the method.

The sample extract is then evaporated and refluxed with phosphoric acid overnight in the presence of cobalt chloride. The solution is basified and the 2,6-dimethylaniline formed is steam distilled using a modification of the steam distillation apparatus of Veith and Kiwus (2). The steam distilled product is derivatized with trichloroacetyl chloride to minimize problems of volatility of 2,6-dimethylaniline. The derivative is cleaned up by alumina column chromatography and analyzed by gas chromatography using an alkali flame ionization detector operating in the nitrogen-specific mode (AFID).

Cottonseed samples are subjected to an additional silica gel column cleanup before the gas chromatographic analysis using the AFID detector.

CIBA-GEIGY Corporation

PAGE 2 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

Selected samples which have interferences in the AFID detector (e.g., cauliflower, onions) are analyzed by gas chromatography - mass spectrometry (GC-MS) in the chemical ionization mode using the M+1 ion at  $m/e$  268. The GC-MS detector is also used for the confirmation of residues analyzed by the AFID detector.

The flow diagram for the method is shown in Figure 1.

### 3.0 APPARATUS

#### 3.1 Extraction and Filtration

- Food chopper, Hobart or equivalent.
- Blender, Waring or equivalent.
- Variable transformer, Powerstat.
- Funnel, 12.5-cm size.
- Filter paper, Whatman 2V, 32-cm.
- Bottle, Boston Round, narrow mouth, 16-oz.
- Mechanical shaker.
- Round bottom flask, 500-ml.
- Condenser, Allihn bulb-type, 500-mm, water cooled.
- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Bottle caps, Poly-Seal lined, 28-mm.

#### 3.2 Partition

- Separatory funnel, 250-ml.
- Round bottom flask, 500-ml.

#### 3.3 Phosphoric Acid Reflux

- Rotary evaporator, Büchi or equivalent.
- Flask, round bottom, 500-ml.
- Condenser, Allihn bulb-type, 500-mm; water cooled.
- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Funnel, 8.5-cm size.
- Thermometer 0-360°C, 2-in. immersion.

## BIOCHEMISTRY DEPARTMENT

084

GREENSBORO, N.C.

PAGE 3 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		APPROVED BY:

### 3.4 Steam Distillation

- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Modified steam distillation apparatus.  
(See Figure 3 for details).

### 3.5 Derivatization

- Separatory funnel, 125-ml.
- Automatic pipette, 100- $\mu$ l, Fisher or equivalent.
- Flask, round bottom, 100-ml.
- Funnel, 6.5-cm size.

### 3.6 Cleanup

- Column, chromatographic, 19-mm i.d., with Teflon stopcock.
- Flask, round bottom 250-ml.
- Graduated concentration tube 20 ml with 24/25 standard taper joint (Kontes K-570050).

NOTE: All the glassware used after the steam distillation step should be rinsed with reagent grade acetone before use to avoid background interference in the GC-AFID detector.

## 4.0 REAGENTS

- Acetonitrile, distilled in glass, Burdick and Jackson.
- Ethyl ether, anhydrous, reagent grade.
- Alumina, basic, (Woelm) W200: Activity Grade V Basic (prepared by addition of 76 ml of water to 324 g of activity grade Super I alumina).
- Phosphoric acid, 85%.
- Cobalt chloride hexahydrate, reagent grade.
- Methanol, reagent grade.
- Hexane, distilled in glass, Burdick and Jackson.
- 25% Sodium hydroxide solution [mixture of equal amounts of distilled water and 50% sodium hydroxide solution (Fisher)].
- Trichloroacetyl chloride, 97%, Aldrich Chemical Company. Catalogue No. 15159-9.
- Dichloromethane, distilled in glass, Burdick and Jackson.
- 5% Aqueous sodium bicarbonate solution.
- Standard metalaxyl.
- Standard N-(2,6-dimethylphenyl)-2,2,2-trichloroacetamide (TCA-DMA).
- Absorbent cotton.
- Silica gel, Davison Chemical Company, Grade H, 100-200 mesh.

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

085

GREENSBORO, N.C.	PAGE 4 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
	EDITION		
	SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

5.0 PROCEDURE5.1 Sample Preparation

A representative sample of 300-400 g of the crop is chopped in a Hobart Food cutter.

5.2 Extraction5.2.1 Extraction of High Moisture Content Crops

NOTE: This extraction is applicable for samples such as potatoes, lettuce, cabbage, fruit crops, melons, cucumbers, onions, etc.

5.2.1.1 Weigh 25 g of the finely chopped sample into a Waring blender jar. Add 250 ml of 20% water/methanol. Blend the sample for 10 minutes using slow speed (use a variable transformer to regulate the speed).

5.2.1.2 Filter the extract through a Whatman 2V filter paper into a 16-oz. bottle (Boston round, narrow mouth).

5.2.1.3 Transfer a 54-ml aliquot (5-g equivalent) to a 500-ml round bottom flask. Concentrate the aliquot to a small volume (approximately 10 ml) using a rotary evaporator (bath temperature 40°C). Proceed to phosphoric acid reflux, Step 5.4.

5.2.2 Extraction of Low Moisture Content Crops

NOTE: This extraction is applicable for samples such as cottonseed, soybeans, grains, dry straw, etc.

5.2.2.1 Weigh 25 g of the finely chopped sample into a 500-ml round bottom flask. Add 250 ml of 20% water/methanol and add a few boiling chips.

## BIOCHEMISTRY DEPARTMENT

088

GREENSBORO, N.C.

PAGE 5 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

5.2.2.2 Place the flask in a 500-ml heating mantle, attach a reflux condenser to the flask and reflux for 2 hours.

5.2.2.3 Allow the solution to cool for about 15 minutes and filter through a Whatman 2V filter paper.

5.2.2.4 For samples other than cottonseed, transfer a 50-ml aliquot (5-g equivalent) to a 500-ml round bottom flask. Concentrate the aliquot to a small volume (approximately 10 ml) using a rotary evaporator (bath temperature 40°C). Proceed to the phosphoric acid reflux, Step 5.4.

5.3 Acetonitrile - Hexane Partition Step (Cottonseed)

5.3.1 For cottonseed samples, transfer a 50-ml aliquot (5-g equivalent) of the extract to a 500-ml round bottom flask. Concentrate the aliquot to a small volume (approximately 10 ml) using a rotary evaporator (bath temperature 40°C). Add 75 ml of 20% water/acetonitrile. Transfer to a 250-ml separatory funnel. Add 50 ml of hexane to the separatory funnel.

5.3.2 Stopper and shake the funnel for 30 seconds, allow the two phases to separate, then drain the aqueous acetonitrile phase (bottom) into a 250-ml erlenmeyer flask. Transfer the hexane phase (top) to another 250-ml separatory funnel.

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

087

GREENSBORO, N.C. \*

PAGE 6 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

5.3.3 Repeat partitioning of the aqueous acetonitrile phase using 50 ml of fresh hexane. Drain the aqueous acetonitrile phase into a 500-ml round bottom flask.

5.3.4 Combine the two hexane phases and back partition using 50 ml of acetonitrile.

5.3.5 Drain the acetonitrile phase and combine with the aqueous acetonitrile phase in the 500-ml round bottom flask. Discard the hexane phase.

5.3.6 Concentrate the combined acetonitrile phases to a small volume (approx. 5-10 ml) using a rotary evaporator. Proceed to the phosphoric acid reflux step (Step 5.4).

#### 5.4 Phosphoric Acid Reflux

5.4.1 Add 100 ml of 85% phosphoric acid, approximately 1 g of cobalt chloride hexahydrate and two or three boiling chips to the flask containing the sample. (Step 5.2.1.3, 5.2.2.4 or 5.3.6).

5.4.2 Place the flask in a 500-ml heating mantle (Note: Do not attach the reflux condenser at this time.) Heat the solution to boiling and monitor the boiling point of the solution using a 0-360°C thermometer, 2-in. immersion, until the temperature reaches  $170^{\circ} \pm 2^{\circ}\text{C}$ .

5.4.3 Remove the thermometer, attach the reflux condenser to the flask and reflux overnight (16 hours).

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

088

GREENSBORO, N.C.

PAGE 7 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

### 5.5 Basification

- 5.5.1 Allow the solution to cool for about 30 minutes, and add 50 ml of distilled water through the top of the condenser using an 8.5-cm funnel.
- 5.5.2 Add 200 ml of 25% sodium hydroxide solution in small increments through the top of the condenser. (Note: Heat is generated by addition of base to the acid solution. A recommended procedure is to add 10 ml of the base to the flask, swirl and let stand for five minutes, then add 20 ml, 30 ml, 40 ml, 50 ml and 50 ml increments of the base, allowing five to ten minutes between each addition and swirling the flask to facilitate mixing). Rinse the condenser with 25 ml of water.

NOTE: Use of gloves during the addition of the base to the flask is recommended for the safe handling of the base.

### 5.6 Steam Distillation

- 5.6.1 Place the flask containing the basified extract in a 500-ml heating mantle and attach the steam-distillation condenser apparatus shown in Figure 3.
- 5.6.2 Add 15 ml of hexane through the top of the steam distillation condenser.
- 5.6.3 Heat the flask to reflux and continue refluxing for one hour. The total heating time is about 1 1/4 hours.

### 5.7 Derivatization Step

- 5.7.1 Withdraw the hexane from the steam distillation apparatus using the solvent withdrawal tube in the side of the apparatus into a 25-ml graduated cylinder.

CIBA-GEIGY Corporation



## BIOCHEMISTRY DEPARTMENT

089

GREENSBORO, N.C.

PAGE 8 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

NOTE: The derivatization of 2,6-dimethylaniline should be done immediately after Step 5.6.3 to prevent losses. The hexane solution may be stored in a freezer if the derivatization must be done at a subsequent time.

- 5.7.2 Filter the hexane phase (top layer) through an 8.5-cm funnel containing absorbent cotton into a 125-ml separatory funnel. Use a pasteur pipet for this transfer. Alternatively, if the sample was kept in the freezer, the aqueous phase will be frozen and the top hexane layer is easily poured into the funnel containing absorbent cotton instead of using a pasteur pipet.
- 5.7.3 Rinse the absorbent cotton and funnel with 15 ml of dichloromethane into the separatory funnel to give a 1:1 mixture of hexane:dichloromethane.
- 5.7.4 Add 100  $\mu$ l of trichloroacetyl chloride to the hexane:dichloromethane solution in the separatory funnel using an automatic pipette and let the solution stand at room temperature for 15 minutes. (Caution: Trichloroacetyl chloride is toxic and corrosive. Hence the addition should be done inside a hood. Also, do not leave the reagent bottle open for an extended period because the reagent is moisture sensitive).
- 5.7.5 Add 25 ml of 5% sodium bicarbonate solution and shake. Draw off the aqueous layer (bottom) and discard. Repeat the partition with an additional 25 ml of 5% sodium bicarbonate solution.
- 5.7.6 Draw off the organic phase (top) into a 125-ml round bottom flask through a funnel containing an absorbent cotton plug. Rinse the funnel using 25 ml of dichloromethane.

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

090

GREENSBORO, N.C.

PAGE 9 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

5.7.7 Evaporate the sample to dryness using a rotary evaporator. (Note: Do not exceed a bath temperature of 30°C. Losses will be encountered if the bath temperature is higher because of the volatility of the derivative).

#### 5.8 Alumina Column Cleanup

- 5.8.1 Fill a chromatographic column (19 mm i.d. containing a glass wool plug at the bottom) with hexane. Measure 30 ml of Grade V alumina using a graduated cylinder and add to the column. Gently tap to remove any trapped air bubbles. (The column is approximately 11 cm in height.)
- 5.8.2 Drain off the hexane until the liquid layer reaches the top of the alumina.
- 5.8.3 Load the sample (Step 5.7.7) onto the column using three 5-ml portions of hexane. Collect the eluate in a 250-ml round bottom flask.
- 5.8.4 Add 150 ml of hexane to the column and collect the eluate in the same flask.
- 5.8.5 Evaporate the hexane eluate to dryness using a rotary evaporator (Note: Do not exceed a bath temperature of 30°C. Losses will be encountered if the bath temperature is higher because of the volatility of the derivative).
- 5.8.6 Add 5 ml of hexane to the flask to dissolve the sample. Pipette a 4-ml aliquot of the sample into a 20-ml sample vial. (Note: An N-Evaporator is not recommended for evaporation because of losses of the derivative encountered by using a stream of air).

or

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

091

GREENSBORO, N.C.

PAGE 10 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

5.8.7 Transfer the sample after the alumina column clean up (Step 5.8.5) to a 20-ml concentration tube using approximately 10 ml of hexane.

5.8.8 Concentrate the sample to 1.0 or 2.0 ml using a rotary evaporator (bath temperature 30°C). The sample is now ready for GC analysis.

5.9 Silica Gel Column Cleanup (for cottonseed samples only)

5.9.1 Fill a chromatographic column (19 mm i.d. containing a glass wool plug at the bottom) with hexane. Measure 30 ml of silica gel using a graduated cylinder and add to the column. Gently tap to remove any trapped air bubbles. (Alternatively prepare the column using a slurry of silica gel in hexane until the column is approximately 4 inches in height).

5.9.2 Drain off the hexane until the liquid layer reaches the top of the alumina.

5.9.3 Load the sample (Step 5.8.5) onto the column using three 5-ml portions of hexane. Add an additional 50 ml hexane to the column. Discard the eluate.

5.9.4 Add 100 ml of 5% ethyl ether/hexane to the column and discard the eluate.

5.9.5 Add 150 ml of 10% ethyl ether/hexane to the column. Collect the eluate in a 250-ml round bottom flask.

5.9.6 Evaporate the sample to dryness using a rotary evaporator. (Bath temperature 30°C).

5.9.7 Add 5 ml of hexane to the flask to dissolve the sample. Pipette a 4-ml aliquot of the sample into a 20-ml sample vial. (Alternatively, concentrate the sample to 1.0 or 2.0 ml using the concentration tube as in Steps 5.8.7 and 5.8.8). The sample is now ready for GC analysis.

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

C92

GREENSBORO, N.C.

PAGE 11 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

## 6.0 GAS CHROMATOGRAPHIC ANALYSIS

The sample in step 5.8.6, 5.8.8 or 5.9.7 is analyzed by gas chromatography using an alkali flame ionization detector operated in the nitrogen-specific mode. The gas chromatographic conditions are given in Table I.

Alternatively, samples are analyzed by gas chromatography-mass spectrometry in the chemical ionization mode by selected ion monitoring (SIM) of the M+1 ion at m/e 268. The gas chromatography mass chromatography (GC-MS) conditions for the analysis are given in Table II.

### 6.1 Standardization

- 6.1.1 Prepare a stock solution containing 100 mg of N-(2,6-dimethylphenyl)-2,2,2-trichloroacetamide (TCA-DMA) in 100 ml of acetone. Serial dilutions should be made with hexane until a working solution containing 0.5 ng/ $\mu$ l is achieved.
- 6.1.2 Dilute the working solution (0.5 ng/ $\mu$ l) to yield standard solutions of 0.2 ng/ $\mu$ l, 0.1 ng/ $\mu$ l and 0.05 ng/ $\mu$ l. Standardize the gas chromatograph, operating under the conditions specified in Table 1 or 2 by injecting 4- to 8- $\mu$ l aliquots of the diluted solutions. This represents a working range of 0.2 ng to 4 ng for the derivative.
- 6.1.3 Determine the peak height or area for the injected standards. Typical chromatograms and the standardization data of TCA-DMA standards using the GC-AFID detector are shown in Figure 4 and Table III, respectively. Typical chromatograms and the standardization data of TCA-DMA standards using GC-MS are shown in Figure 5 and Table IV, respectively.
- 6.1.4 Construct a standard curve, plotting detector response (peak height or area) versus nanograms injected. Typical standard curves for GC-AFID and GC-MS are presented in Figures 6 and 7, respectively. Alternatively, enter the standardization data into an appropriate electronic calculator (e.g., Texas Instrument Model TI55) to calculate a least square standard curve.

CIBA-GEIGY Corporation

PAGE 12 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

## 6.2 Detection of Sample Residues

- 6.2.1 Inject a 4-8  $\mu$ l aliquot of the sample in Step 5.8.6, 5.8.8 or 5.9.7 into the gas chromatograph. Make appropriate dilutions of the sample to have the sample peak height within the range of the standard curve. Compare peak heights or areas of unknown samples with the standard curve or enter into the least square program of the calculator to determine the amounts of the derivative in the aliquot injected.

Typical chromatograms of selected samples using GC-AFID detection are shown in Figures 8-9. Typical chromatograms of selected samples using GC-MS are shown in Figure 10.

- 6.2.2 Calculate residue results as ppm equivalents of metalaxyl using the following equation:

$$\text{PPM} = \frac{\text{Amount TCA-DMA found (ng)}}{\text{mg crop injected}} \times 1.053 + R$$

where R is the recovery factor determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.0, etc.).

The factor 1.053 is used to convert residues of TCA-DMA found into metalaxyl equivalents.

## 7.0 DISCUSSION

- 7.1 The percent recovery of metalaxyl from potato tubers fortified at 0.05 to 1.0 ppm levels ranged from 60 to 83% with an average of  $72 \pm 10$  percent ( $n = 10$ ). Recovery data for rotational crops analyzed (winter wheat, soybeans, corn, lettuce, sweet potatoes, sugar beets, and rye) in the range of 0.05 to 1.0 ppm showed an average recovery of  $72 \pm 11$  percent ( $n = 24$ ).

## BIOCHEMISTRY DEPARTMENT

094

GREENSBORO, N.C.

PAGE 13 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

7.2 Cucurbit samples (honeydew, cucumbers, watermelons, cantaloupe) fortified at 0.05 to 0.40 ppm showed recoveries ranging from 53 to 95% with an average recovery of  $72 \pm 11\%$  ( $n = 18$ ).

7.3 Cole crops (broccoli, cabbage, cauliflower) fortified at 0.05 to 0.50 ppm showed recoveries ranging from 42 to 79% with an average recovery of  $59 \pm 11\%$  ( $n = 24$ ).

7.4 An interference peak was seen in selected samples of cole crops (cabbage, cauliflower and onions) in the gas chromatographic analysis using the AFID detector. These samples analyzed by gas chromatography - mass spectrometry showed no interference at the screening level of 0.05 ppm.

#### 8.0 REFERENCES

1. AG-330: "The determination of CGA-48988 and its metabolites in tobacco as 2,6-dimethylaniline using phosphoric acid reflux."
2. G. D. Veith and L. M. Kiwus, "An Exhaustive Steam-Distillation and Solvent-Extraction Unit for Pesticides and Industrial Chemicals," Bulletin of Environmental Contamination and Toxicology, Vol. 17, 1977, p. 631-636.

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

095

GREENSBORO, N.C.

PAGE 14 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

TABLE I. GAS CHROMATOGRAPHIC CONDITIONS FOR GC-AFID ANALYSIS

<u>Instrument</u>	Tracor 200 equipped with an alkali flame ionization detector (Perkin-Elmer)
<u>Column Packing</u>	3% Dexsil 300 on Gas Chrom Q (80/100 mesh)
<u>Column</u>	Pyrex 4' x 4 mm i.d.
<u>Temperatures</u>	
Column	155°C
Injector	265°C
Detector	255°C
<u>Gas Flows</u>	
He carrier	60 ml/min.
H <sub>2</sub> reaction gas	3.0 ml/min. (regulated)
Compressed air	100 ml/min.
<u>Attenuation</u>	1 x 8
<u>Bead Current Setting</u>	745
<u>Minimum Detection Limit</u>	0.5 nanogram
<u>Volumes Injected</u>	4-8 µl
<u>Chart Speed</u>	1 cm/minute
<u>Retention Time</u>	3.7 minutes

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

098

GREENSBORO, N.C.

PAGE 15 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

TABLE II: INSTRUMENT CONDITIONS FOR GC-MS ANALYSISInstrument

Finnigan Model 3200 gas chromatograph - Mass Spectrometry operating in the chemical ionization mode with methane as the reactant and carrier gas. (Between the column and the MS interface, a "T" equipped with a toggle valve to a vacuum line is used to vent large volumes of solvent without impairing the functions of the mass spectrometer).

Carrier gas:

Methane flow adjusted to give 1,000  $\mu$  pressure in the ion source.

Temperatures

Column	165°C
Inlet	230°C
Interface	250°C
Transfer	250°C
MS Manifold	90°C

MS Settings

Electron energy	120 eV
Emission	0.50 ma
Ion energy	15V (volts)
Collector	30 (volts)
Lens	40 (volts)
Extractor	10V (volts)
Electron multiplier	2.0 (KV)
Mass range	268.0 $\pm$ 0.2 amu (scan range 0.2 amu)
Sensitivity	10 <sup>-8</sup>
Ion current	
integrator	100
Recorder	1.0 V
Chart speed	1 cm/sec.
Vent time	1.0 min.
Retention time	3.5 min.
Minimum detection	0.2 ng
limit	

CIBA-GEIGY Corporation



## BIOCHEMISTRY DEPARTMENT

097

GREENSBORO, N.C.

PAGE 16 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

TABLE III: TYPICAL STANDARD CURVE FOR GC-AFID ANALYSIS

Amount TCA-DMA Injected (Nanograms)	Peak Height (cm)
1.0	1.9
8.0	15.0
2.0	2.9
4.0	6.8
0.2	0.3
1.0	1.4
2.0	3.1
4.0	6.8
8.0	15.3

See Figure 4 for plot.

Regression Data for the standard curve

n=9 Correlation coefficient 0.9981 slope: 1.910  
Intercept: -0.4991

CIBA-GEIGY Corporation

## BIOCHEMISTRY DEPARTMENT

098

GREENSBORO, N.C.

PAGE 17 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

APPROVED BY:

TABLE IV. TYPICAL STANDARD CURVE FOR GC-MS ANALYSIS

Amount TCA-DMA Injected (Nanograms)	Peak Height (cm)
2.0	12.9
0.25	2.0
0.5	3.9
1.0	7.0
0.5	3.5
1.5	10.2
0.25	1.8
0.5	3.2

See Figure 5 for plot.

Regression data for the standard curve

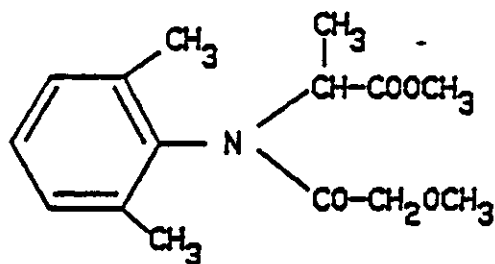
n=8 Correlation coefficient: 0.9980 Slope: 6.398

Intercept: 0.3643

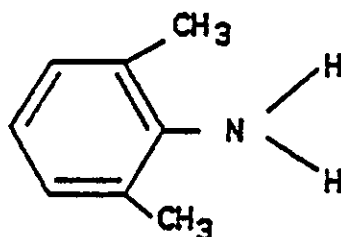
CIBA-GEIGY Corporation

PAGE 18 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		

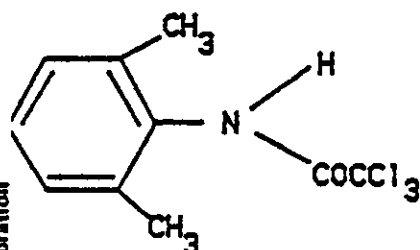
APPROVED BY:

Metalaxyl, CGR-48988

N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)-alanine  
methyl ester  
 $C_{15}H_{21}O_4N$

CGR-72649

2,6-dimethylaniline

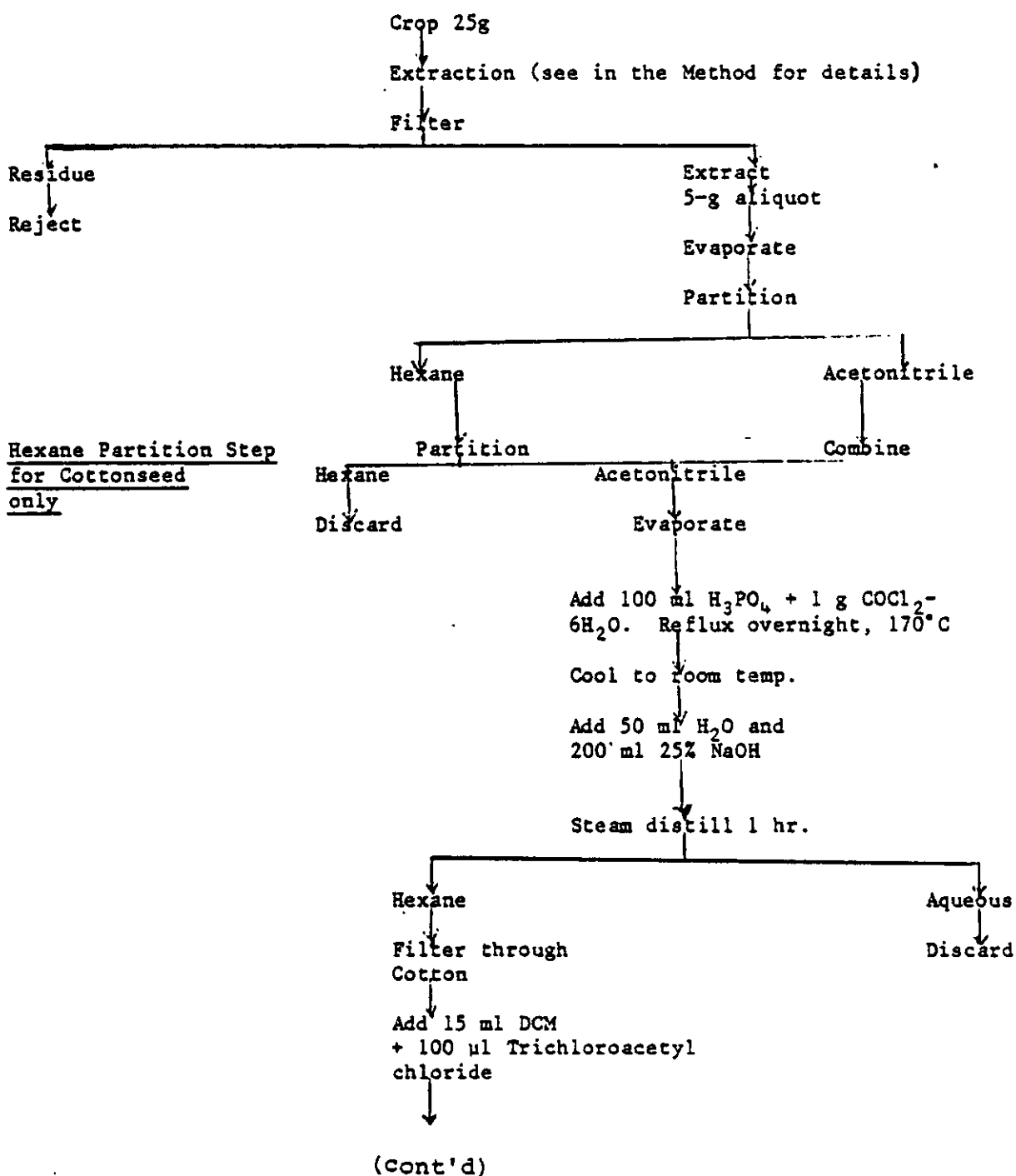
TCR-DMA

N-(2,6-Dimethylphenyl)-  
2,2,2-trichloroacetamide

Figure 1: CHEMICAL NAMES AND STRUCTURES

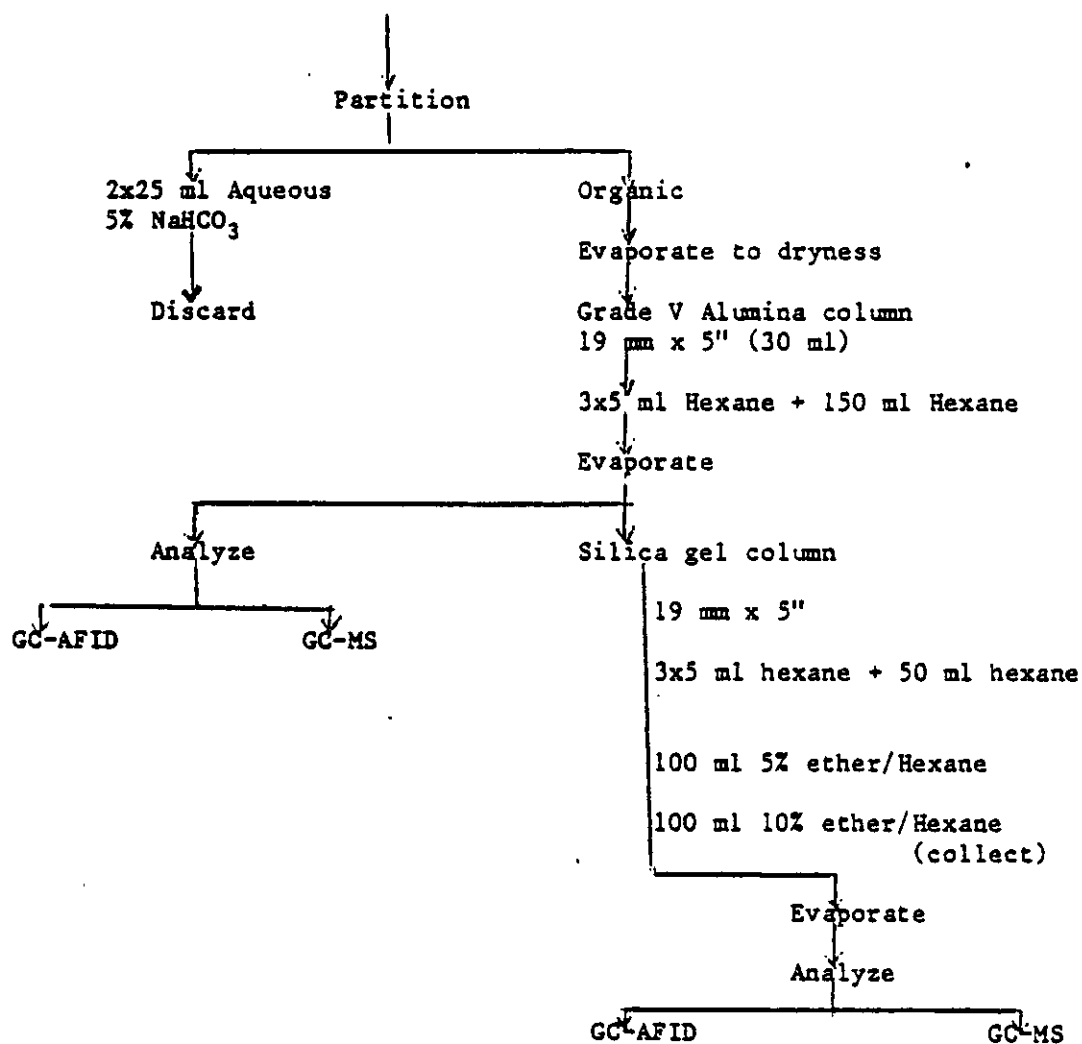
PAGE 19 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

FIGURE 2: FLOW DIAGRAM OF THE ANALYTICAL PROCEDURE FOR THE DETERMINATION OF METALAXYL AND ITS METABOLITES IN CROP



PAGE 20 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

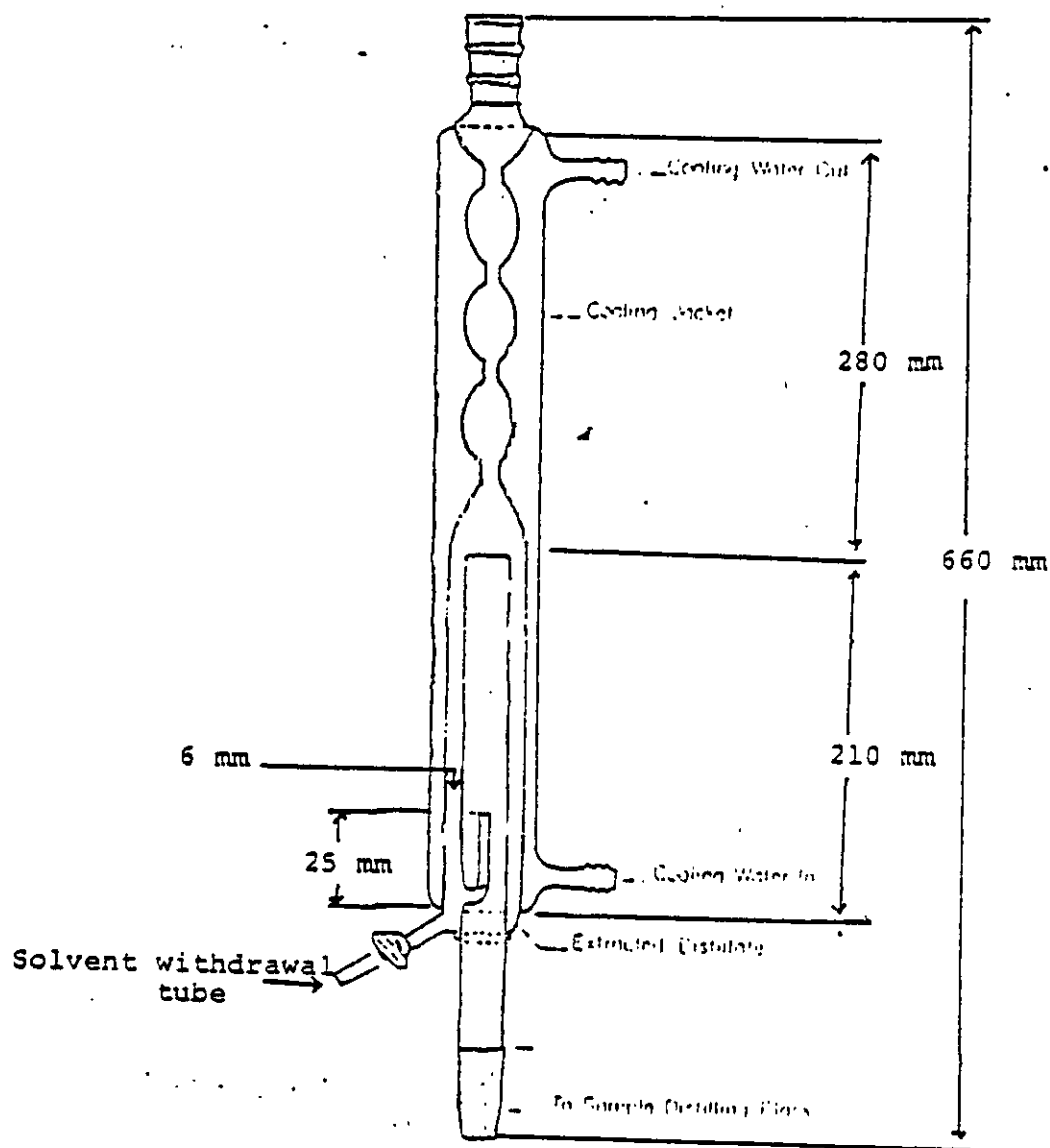
FIGURE 2: FLOW DIAGRAM OF THE ANALYTICAL PROCEDURE FOR THE DETERMINATION OF METALAXYL AND ITS METABOLITES IN CROP (Continued)



NOTE: Silica gel column cleanup for cottonseed sample only.

PAGE 21 of 28	METHOD No. AG-348	SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN CROPS AS 2,6-DIMETHYLANILINE
EDITION		
SUBMITTED BY: K. Balasubramanian		
		APPROVED BY:

FIGURE 3: EXHAUSTIVE STEAM-DISTILLATION AND SOLVENT-EXTRACTION APPARATUS



Note: The length of the tube (25 mm) and the space between the jackets (6 mm) are important. These dimensions assure that approximately 15 ml of organic solvent is contained in the well between the jackets.

FIGURE 4: TYPICAL GAS CHROMATOGRAPHY OF TCA-DMA STANDARDS  
USING GC-AFID

3% Dexsil  
GC-AFID Detector

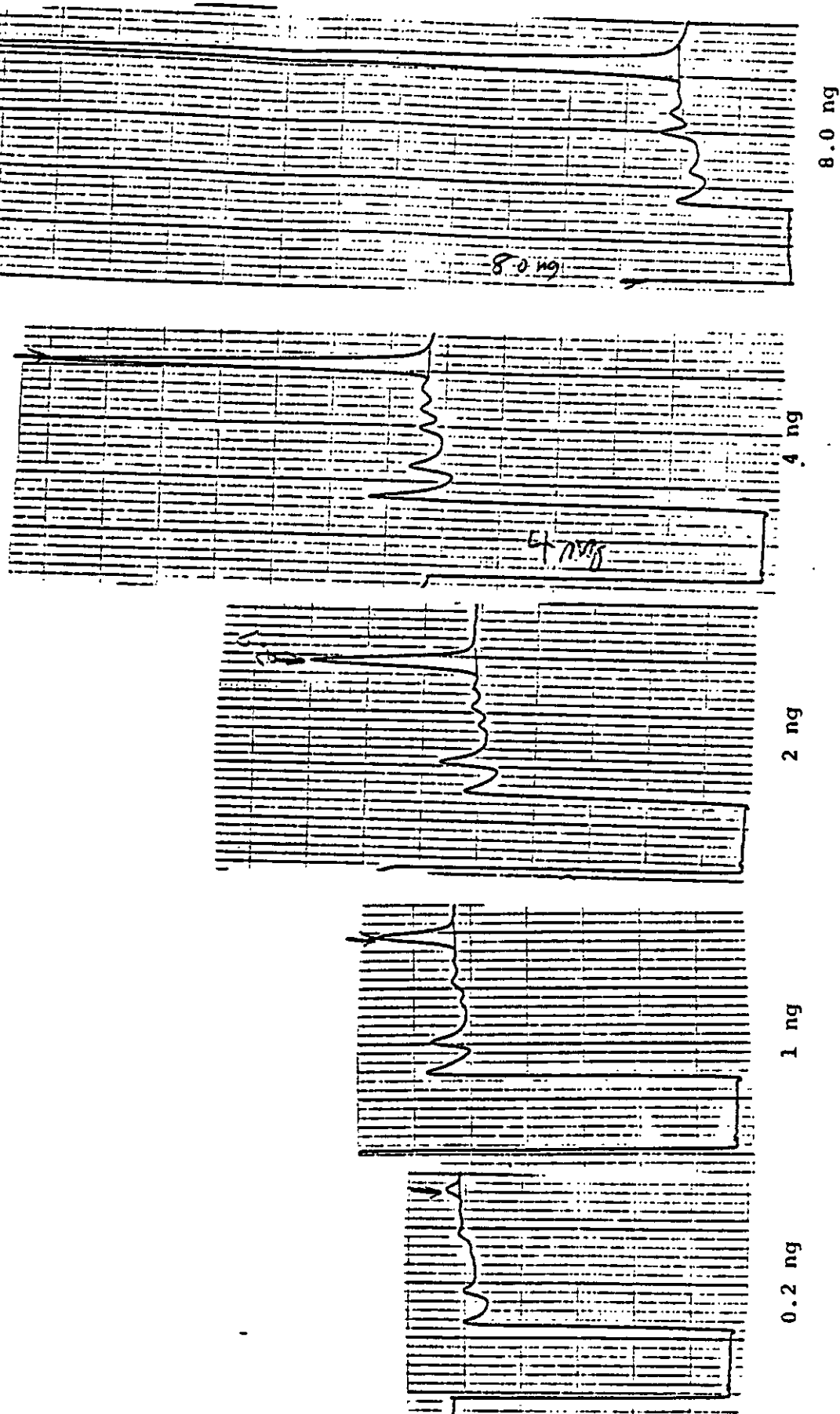


FIGURE 5: TYPICAL GAS CHROMATOGRAPHY OF  
TCA-DMA STANDARDS USING GC-MS

3% Dexsil  
GC-MS, SIM m/e 268

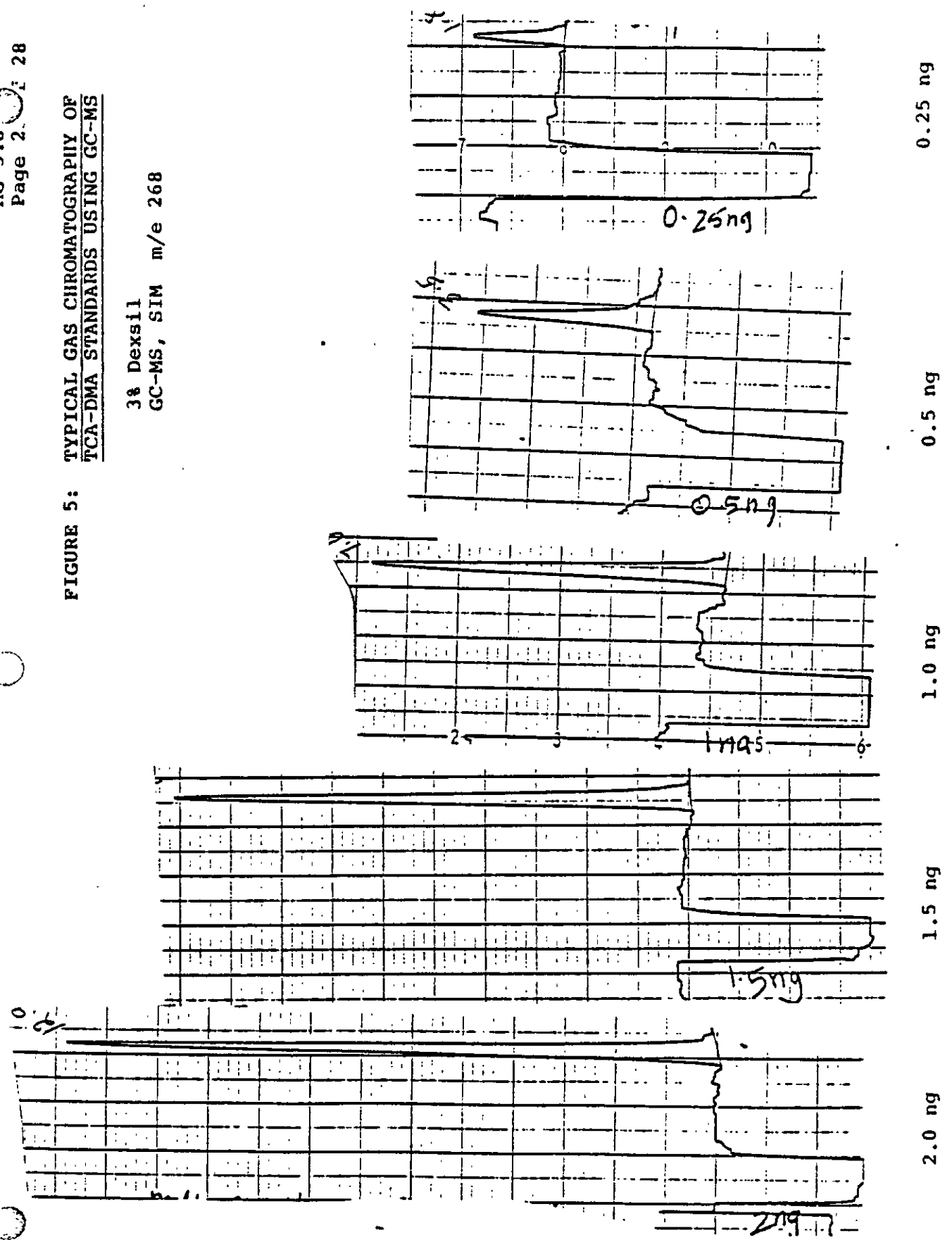




FIGURE 6: TYPICAL STANDARD CURVE OF TCA-DMA BY ALKALI  
FLAME IONIZATION DETECTOR (SEE TABLE III FOR DATA)

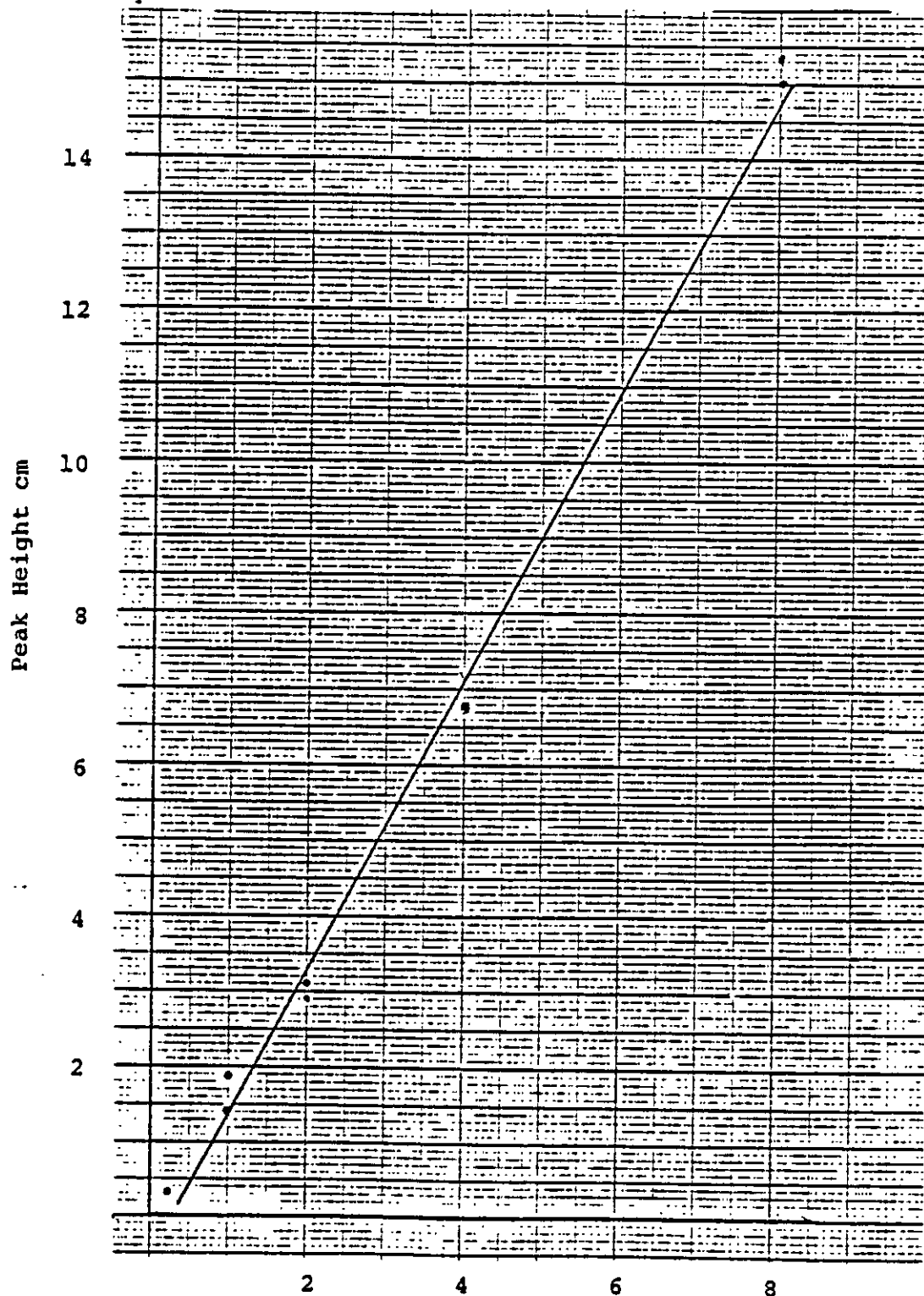
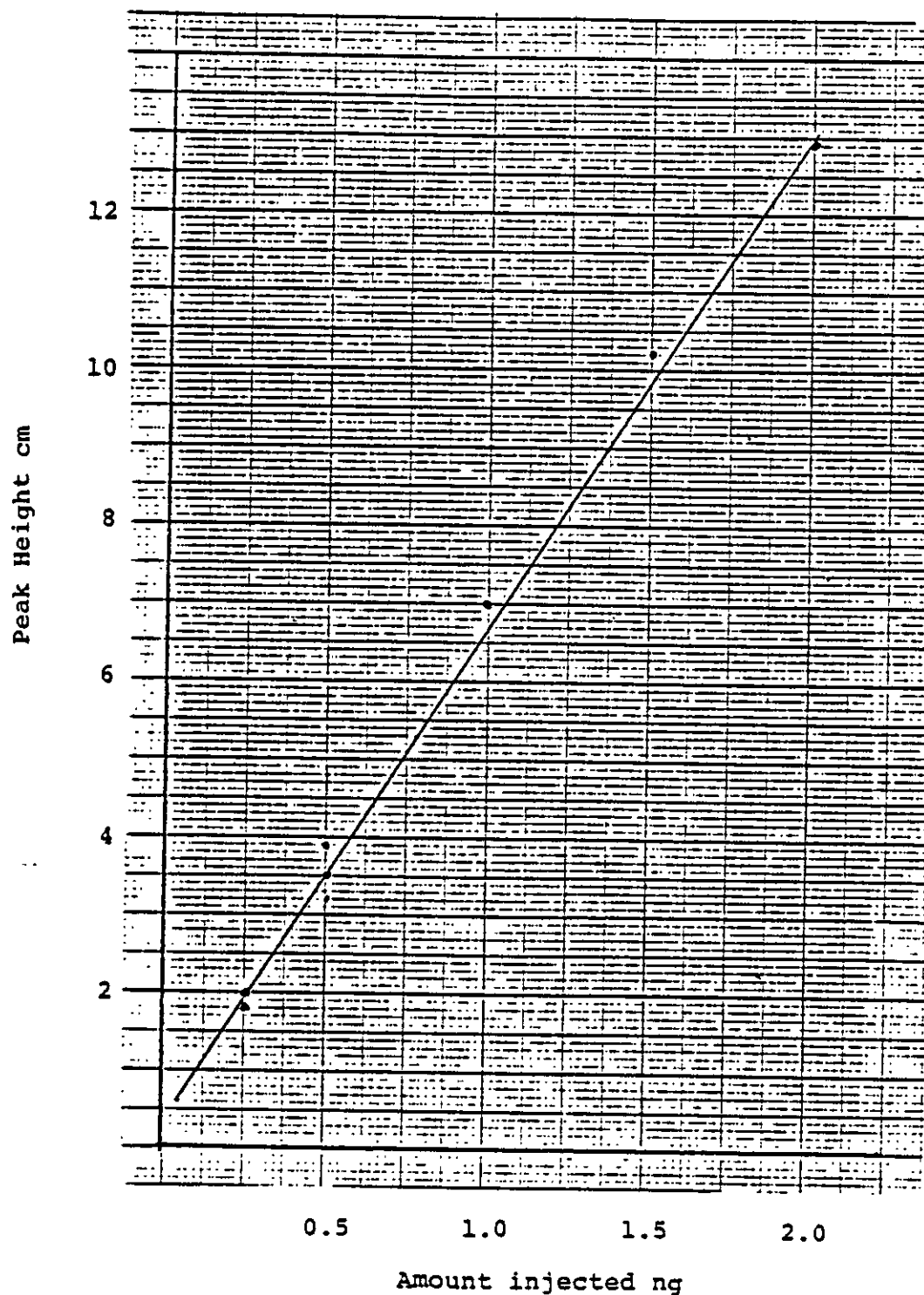
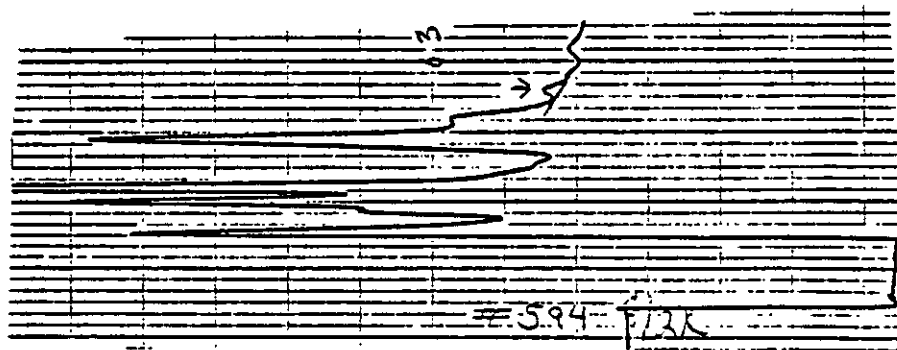


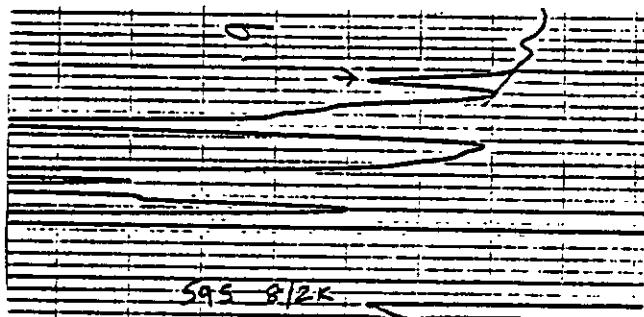
FIGURE 7: TYPICAL STANDARD CURVE OF TCA-DMA BY GAS  
CHROMATOGRAPHY - MASS SPECTROMETRY (SEE  
TABLE IV FOR DATA)



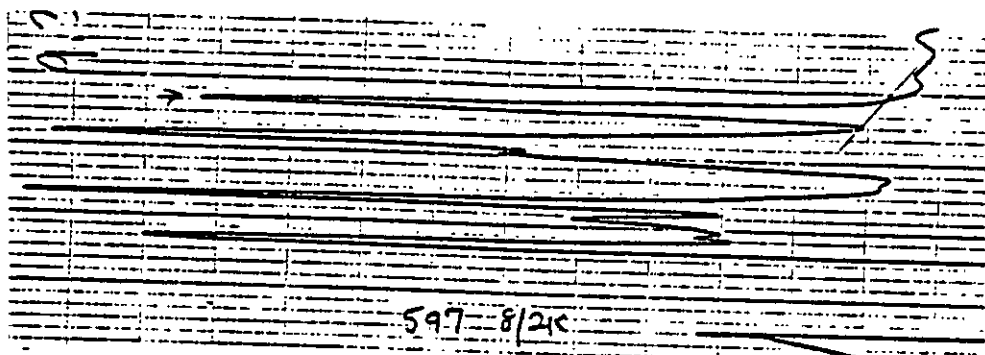
AG-A: 5878 II



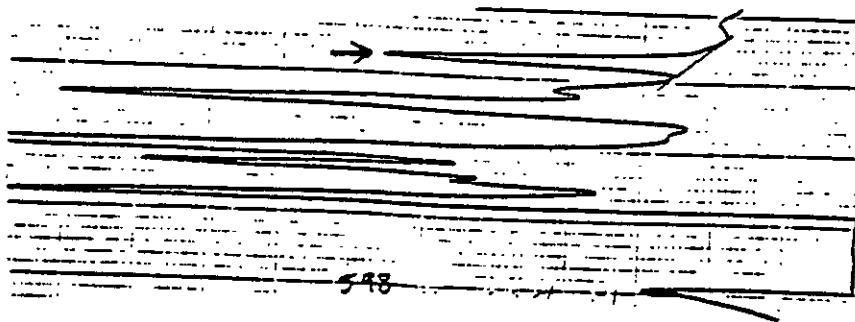
Check 1-2-A  
20 mg injected  
Found 0.4 ng  
<0.05 ppm



Check + 0.10 ppm  
Metalaxyl  
20 mg injected.  
Found 1.4 ng  
Found 0.073 ppm  
73% rec.



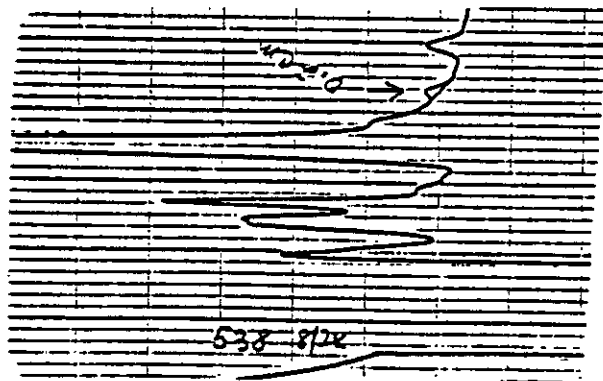
Check + 0.50 ppm  
Metalaxyl  
20 mg injected  
Found 7.3 ng  
0.38 ppm  
77% rec.



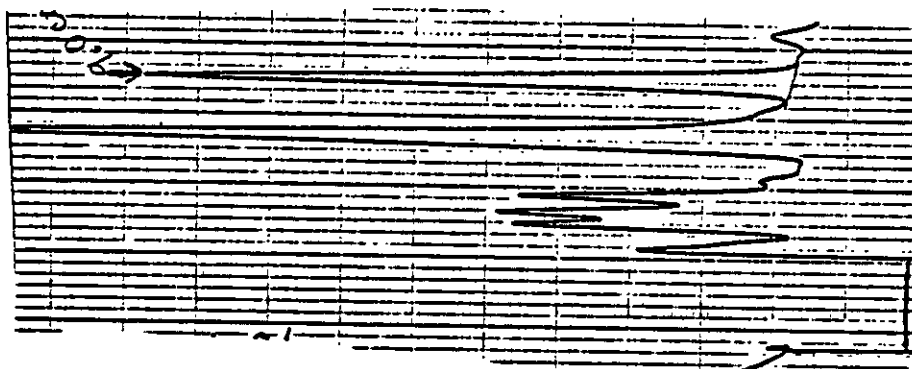
1X treated 2-2-A  
20 mg injected.  
Found 3.6 ng  
0.19 ppm

FIGURE 9: TYPICAL GC-AFID SCANS FOR ANALYSES OF CUCURBITITS

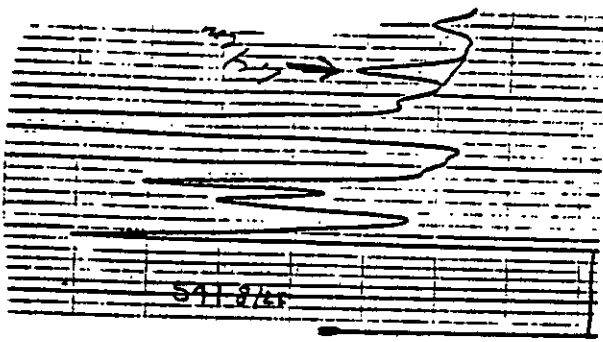
Sample: Honeydew melons  
AG-A 5681



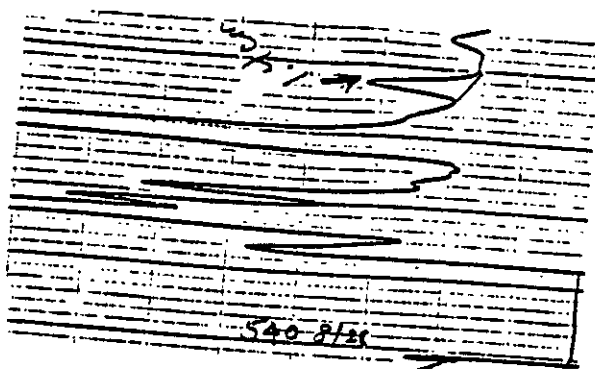
Check 1-1-AB  
20 mg injected  
Found <0.2 ng  
<0.05 ppm



Check + 0.40 ppm  
20 mg injected  
Found 4.8 ng  
0.25 ppm  
63% rec.



1X treated 2-1-AB  
20 mg injected  
Found 1 ng  
0.05 ppm



2X treated 3-2-AB  
20 mg injected  
Found 1 ng  
0.05 ppm

AG-A 5726

